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Investigation of autoantibody positivity in patients with spondyloarthritis

Seropositive spondyloarthritis frequency

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Abstract
Aim: Although ankylosing spondylitis (AS) and undifferentiated spondyloarthritis (uSpA) are included in seronegative spondyloarthropathy (SpA) classification, autoantibody positivity has been observed in the clinical practice. However, there is no study evaluating the frequency of autoantibody positivity in this group of patients in the literature. The objective of this study is to evaluate the frequency of serologic markers in AS and uSpA, and to compare it with the normal population.
Material and Methods: In total, 1486 patients with spondyloarthropathy were included in the study. Autoantibodies used in the diagnosis of rheumatic diseases were evaluated in patients. In addition, 149 healthy volunteers without known chronic diseases were selected as the control group.
Results: Among 1486 patients with SpA, 950 subjects (63.9%) had uSpA and 536 subjects (36.1%) had AS. Autoantibody positivity was observed in 96 patients. In this seropositive patients group, accompanying systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS) were observed in 6 patients (6.3%) and 23 patients (24%), respectively.
Discussion: There was no significant difference in terms of autoantibody positivity between patients with SpA and healthy controls. Autoantibody positivity was higher in the uSpA subgroup with female predominance and peripheral joint involvement.

Keywords
Spondyloarthritis; Ankylosing spondylitis; Undifferentiated spondyloarthritis; Autoantibody positivity

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Introduction

Spondyloarthropathies (SpA) comprise a group of chronic, inflammatory diseases sharing common clinical, radiographic, and genetic features [1]. SpA includes undifferentiated spondyloarthritis (uSpA), ankylosing spondylitis (AS), reactive arthritis (ReA), psoriasis and psoriatic arthritis (PsA) related to spondyloarthritis, enteropathic arthritis, and juvenile-onset spondyloarthritis [2].

Though commonly used serological tests for diagnosis of autoimmune rheumatic diseases such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibodies (ANA), extractable nuclear antigens (ENA) panel tests are assumed to be negative in SpA, this concept has gradually been losing its value, since recent studies have pointed out their coexistence especially in PsA patients [3,4]. Although AS and uSpA are thought to be seronegative, in clinical practice some degree of autoantibody positivity could be seen in this group of patients. There is no comprehensive study in the literature regarding the prevalence of autoantibodies in this patient group. The aim of this study is to determine the frequency of some autoantibodies used in rheumatology (RF, anti-CCP, ANA, ENA panel tests, and anti-dsDNA) in patients with AS and uSpA, and to compare these results with healthy control group. In addition, the clinical and demographic characteristics of the autoantibody positive SpA group will be examined and the frequency of other rheumatic diseases accompanying SpA will be evaluated.

Material and Methods

This study has been carried out on patients applied to the rheumatology clinic of Ankara Ataturk Training and Research Hospital. The patients were evaluated according to the Modified New York [5], and ESSG criteria [6], and 536 subjects were diagnosed as AS and 950 subjects as uSpA. The age range of these patients included in the study group was 18-70 years and there was no active infection, organ failure, malignancy or pregnancy. American College of Rheumatology (ACR) Classification Criteria was used for Systemic Lupus Erythematosus [7], and Sjögren syndrome [8]. In addition, 149 healthy volunteers without any known chronic disease were chosen as the control group. Informed consent forms were obtained from the patients. Ethical approval was obtained by the Ethics Committee of Yildirim Beyazit University, Ankara Ataturk Training and Research Hospital.

All serological evaluations of the patients were performed on the day of diagnosis and before the start of drug treatment. Venous blood samples were taken from the study and control subjects, and serologic tests were performed on the same day at Atatürk Training and Research Hospital Central Laboratory. Complete blood count, biochemical parameters (liver and kidney function tests, electrolytes), c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), RF, ANA, anti-CCP, anti-ds DNA, ENA panel and HLA B-27 were examined, and the results were evaluated. ENA panel was evaluated by the Western blot method (if ANA was positive or sicca symptoms were present), RF by nephelometric method, anti-CCP by electrochemiluminescence immunoassay (ECLIA) method, ANA and anti-dsDNA by spectrophotometric (Micro Elisa) method. Upper limits of RF, anti-CCP and anti-ds DNA were adopted as 20 IU/ml, and the values higher than 20 IU/ml were considered as positive. In the positive ANA titer, the limit value was 1/100. Sacroiliac X-ray and MRI were also obtained for the diagnosis of SpA of patients.

Statistical Analysis

The results were analyzed by the SPSS software package (SPSS for Windows, version 15.0, SPSS, Chicago, Ill). The relationship between the variables was investigated by the Chi-Square test. P- value less than 0.05 was accepted as statistically significant.

Results

Among the 1486 patients with spondyloarthritis (SpA), 950 subjects (63.9%) had uSpA and 536 subjects (36.1%) had AS. Autoantibody positivity was observed in 96 patients. In this autoantibody positive group, accompanying systemic lupus erythematosus (SLE) was observed in 6 patients (6.3%) and Sjögren's syndrome (SS) in 23 patients (24%). Most of the patients were female (87.5%). Some clinical and laboratory characteristics of autoantibody positive SpA patients and healthy controls are shown in Table 1.

As seen in Table 1, most of the study patients were presented with peripheral SpA features (88.5%), mostly have uSpA (89.6%), were usually HLA B27 negative (56.3%), and frequency of recent infection was about 30%. Due to autoantibody positivity and accompanying autoimmune diseases, frequency of anti-TNF treatment use was low (1%) in this group.

Assessment of autoantibody positivity rates in the study group was as follows: 96 of 1486 patients had autoantibody positivity, among this 1486 SpA patients, 34 patients (2.3%) had RF positivity, 13 patients (0.9%) had anti-CCP positivity, 75 patients (5%) had ANA positivity, 8 patients (0.5%) had anti-ds DNA positivity, and 19 patients (1.3%) had positivity for one of the other ENA autoantibodies (7 patients for SS-A and SS-B, 7 patients for SS-A, 2 patients for SS-B, 2 patients for Scl 70, and 1 patient for Sm-RNP) (Table 2).

There was no difference between study and control groups regarding the RF positivity ($p > 0.05$). However, most of the RF positive patients were in uSpA subgroup (29 patients, 3%), while 5 of them (0.9%) were in the AS subgroup. The difference between the subgroups was statistically significant ($p = 0.033$, see Table 2). The frequency of anti-CCP positivity was not significant between the study and control groups. Anti-CCP positivity was also more frequent in uSpA subgroup, similar to RF. (Tables 2, 3).

The frequency of ANA positivity in the SpA group was lower than the control group, but it was not statistically significant. ($p > 0.05$, see Table 2). The frequency of ANA positivity was 68 (7.1%), 7 (1.3%), and 9 (6%) in uSpA, AS, and control groups, respectively. The frequency of ANA positivity of uSpA subgroup was statistically higher than the AS subgroup ($p < 0.001$) and the control group ($p = 0.015$, see Table 3). The frequency of Anti-ds DNA positivity was not statistically significant between the SpA patients and healthy subjects. However, although statistically nonsignificant, it was higher in uSpA group than those of the AS group. In the AS subgroup, there was no subject having anti-ds DNA positivity. On the other hand, eight patients (0.8%) in uSpA subgroup and only 1 subject (0.7%) in the control group had

Table 1. Some clinical and laboratory characteristics of SpA patients and healthy controls

Variables	SpA patients (n = 96)	control group (n = 149)
Age (range)	50 (18-70)	45 (34-63)
Female, n (%)	84 (87.5%)	95 (63.8%)
Male, n (%)	12 (12.5%)	54 (36.2%)
uSpA groups, n (%)	86 (89.6%)	
AS groups, n (%)	10 (10.4%)	
Symptom Duration [month] (range)	48 (6-360)	
Diagnosis Duration [months] (range)	24 (2-190)	
Family History, n (%)	19 (19.8 %)	
Concomitant rheumatic disease history n (%)		
SLE	6 (6.3%)	
Sjogren syndrome	23 (24%)	
Used Drug		
NSAID	4 (4.2 %)	
DMARD	28 (29.2 %)	
DMARD and NSAID	60 (62.5 %)	
Anti-TNF	1 (1 %)	
HLA B27 positivity		
Negative	54 (56.3 %)	
Positive	10 (10.4 %)	
Unknown	32 (33.3 %)	
Axial Involvement	93 (96.9 %)	
Peripheral Involvement	85 (88.55)	
Enthesopathy	57 (59.4%)	
Eye Involvement		
Uveitis	2 (2.1%)	
Dry eye	42 (43.8%)	
Sacroiliac MRI		
Normal	24 (25%)	
Sacroiliitis	62 (64.6%)	
Unknown	10 (10.4%)	

Table 2. Comparisons of autoantibody positivity between spondyloarthritis patients and control group (*)

	Spondyloarthritis patients (n = 1486)		Control (n = 149)		P
RF positivity (%)	34	(2.3%)	6	(4%)	>0.05
Anti-CCP positivity (%)	13	(0.9%)	0	(0%)	
ANA positivity (%)	75	(5%)	9	(6%)	
Anti-ds DNA positivity (%)	8	(0.5%)	1	(0.7%)	
ENA positivity (%)	19	(1.3%)	1	(0.7%)	

(*) with the Chi-square test

anti-ds DNA positivity. The difference between AS subgroup and the control group was also not statistically significant (p> 0.05, see Table 3).

There was also no patient having ENA positivity in AS subgroup. Nineteen patients (2%) in subgroup uSpA and 1 subject (0.7%) in the control group showed ENA positivity. ENA positivity was not statistically significant between healthy and study group, but was significantly higher in the uSpA subgroup than AS (p = 0.003, see Table 3). Anti-SS-A and SS-B positivity in ENA panel was especially remarkable.

Table 3. Comparison of autoantibody positivity of uSpA, AS and control groups (*)

Positivity (%)	uSpA (n = 950)		AS (n = 536)		Healthy control (n = 149)		P		
							uSpA-AS	uSpA-HG	AS-HG
RF	29	(3%)	5	(0.9%)	6	(4%)	0.033	1	0.092
Anti-CCP	11	(1.2%)	2	(0.4%)	0	(0%)	0.285	0.401	1
ANA	68	(7.2%)	7	(1.3%)	9	(6%)	<0,001	0.015	<0,001
Anti-ds DNA	8	(0.8%)	0	(0%)	1	(0.7%)	0.118	1	0.3
ENA	19	(2%)	0	(0%)	1	(0.7%)	0.003	1	0.8

(*) with the Chi-square test , HG : Healty Group

Discussion

In this study conducted with 1486 patients, it was found that autoantibody positivity could be seen in SpA patients, however, the frequency of autoimmune disease was similar to healthy individuals. There are several studies in the literature with a small number of patients. However, there was no study in which all autoantibodies were screened and the patients with SpA were divided into AS and uSpA subgroups. Therefore, we believe that our study will contribute to the literature due to a large number of patients and the parameters examined.

In our study, 96 of 1486 SpA patients had autoantibody positivity against ANA, RF, anti-dsDNA, anti-CCP or ENA. Six of 96 patients with autoantibodies were accompanied by SLE (0.4 - 6 \ 1486) and 23 with SS (1.5 - 23 \ 1486). Autoantibody positivity was significantly higher in the uSpA subgroup than in the AS group. Therefore, the uSpA group may be more likely to have other autoimmune diseases.

Oscar et al. investigated the incidence of autoimmune disease in 148 SpA patients and the SS incidence was 1.4% (2/148), similar to our study. On the other hand, in the same study, the presence of SpA was investigated in 1077 patients with different autoimmune diseases such as RA, SLE and SS, and it was detected in only 5 patients (0.46%) with a similar frequency to the healthy control group [9].

In a study conducted with a healthy Turkish population SS incidence have found a rate of 1.56%. This ratio is consistent with the results of our study [10]. In another study, the frequency of sacroiliitis was investigated in patients diagnosed with primary Sjögren and was not different from the healthy control group [11]. Although the number of cases is different, studies have shown that the incidence of SS is similar in patients with SpA to the healthy control group.

There was no study in the literature covering the whole group of patients with spondyloarthropathy and more than a thousand patients. In our study, 13 patients with SpA had anti-CCP positivity and 11 were in the uSpA group; however, it was not statistically significant. Anti-CCP positivity was also associated with peripheral joint involvement. (88.5%) In the study by Lopez and Kim, anti-CCP positivity of the patients with AS was found to be 5.5% and 4%, respectively, and positivity was associated with peripheral joint involvement [12,13]. Similar studies with patients with PsA have shown that anti-CCP may be positive, leading to erosive disease and dactylitis by increasing disease

activity [14-17]. In our study, none of the 149 healthy controls had anti-CCP positivity, but Popescu found 1.4% of 147 healthy individuals [14]. All of these studies show that anti-CCP may be positive in patients with seronegative SpA, and positivity will increase disease activity.

It has been reported that rheumatoid factor (RF) may be positive in rheumatic diseases and non-rheumatic diseases and may be seen in 10% of healthy individuals [18]. There is only an old study in the literature evaluating RF positivity in patients with AS. In this study, 14 out of 112 patients were RF positive (12.5 %) [19]. In our study, the RF frequency was less and there was no difference between the SpA group and the control group. However, it was significantly more frequent in patients with uSpA than in patients with AS. In Popescu and Singh's studies, RF positivity was found in patients with PsA but was not different from the control group [14,20].

Anti-nuclear antibodies (ANA) are autoantibodies formed against cytoplasmic or nucleolar self-antigens [21]. ANA test may be positive in systemic autoimmune diseases (SLE, SSc, RA, etc.), in some organ-specific autoimmune diseases, in some patients using some drugs such as anti-TNF, cyclosporine and isoniazid, in elderly subjects, and in some infections. ANA positivity rate is 1 to 5 percent in healthy subjects [22].

In our study, there was no difference between patients and healthy group in terms of ANA, Anti-Ds DNA, ENA positivity. However, ANA and ENA positivity was significantly more frequent in uSpA patients than in AS patients and control group. Especially ANA and ENA positivity were statistically significant. In our study, all of the serologic assessments were performed before the initiation of medication. The results were not affected by drug treatment.

In Hoxha's study, only 1 of 30 AS patients had ANA positivity [23], whereas in Gonnet-Gracia's study, 27% of 61 AS patients had ANA positivity and 2% had anti-ds DNA positivity [24]. In another study ANA positivity was found in 47% of 23 patients with PSA [25]. In all studies, ANA positivity was increased after treatment with anti-TNF alpha. [23, 24, 25]

It has been observed that SpA patients with autoantibody positivity were usually in uSpA subgroup, they were generally female, and peripheral joint involvement in these patients was common. RF, anti-CCP, ANA, anti-dsDNA, and ENA panel tests may be positive in SpA patients, and some other autoimmune diseases may co-exist.

As a conclusion, commonly used autoantibodies might be found positive in patients with SPA. Routine screening for these autoantibodies is not recommended and might be performed in those with relevant symptoms. In patients with rheumatic complaints and autoantibody positivity, a diagnosis of SpA should not be overlooked.

In this study, only the patients with seropositivity were evaluated in detail.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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